# Sequences

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Current Topics in Genome Analysis *Tuesday March 23, 1999* 

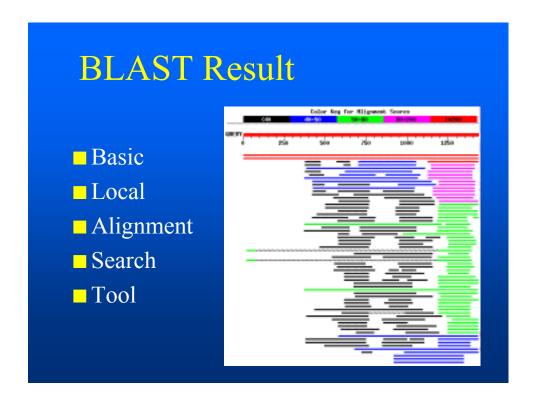
### **Outline**

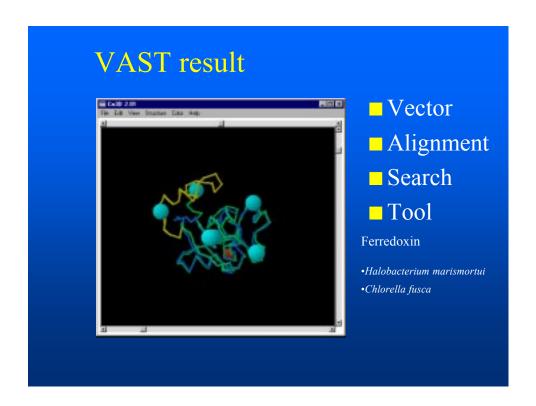
- What is Bioinformatics
- What is the primary sequence data
- What is GenBank
- Tools for submissions of sequences
- Updates: finishing the job

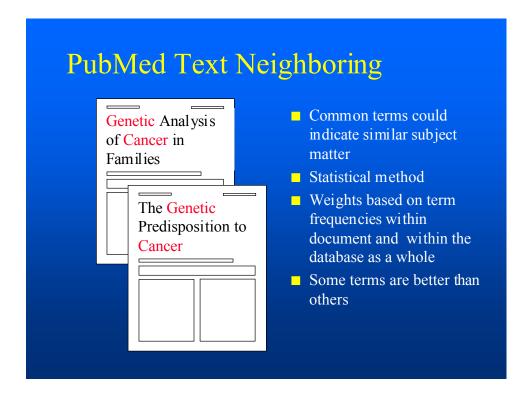
### Computational Biology

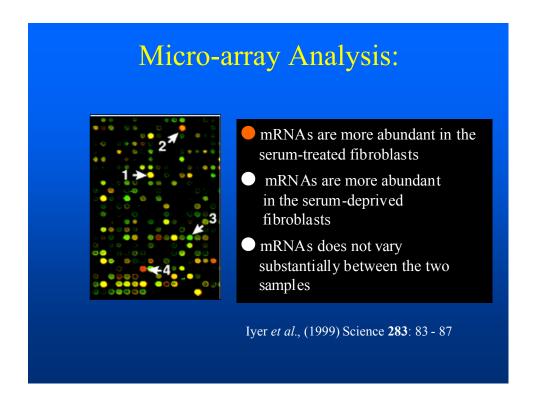
- "New" Field of Science where mathematics, computer science and biology combine together to study and interpret genomic and proteomic information.
- CB will provide the tools for fully talking advantage of the HGP (est. 2003) as well as all of the other genome projects.
- CB will position its users at the head of the pack in any race for drug target discovery as well as improving healthcare worldwide.

Bioinformatics is about bringing biological themes together with the help of computer tools









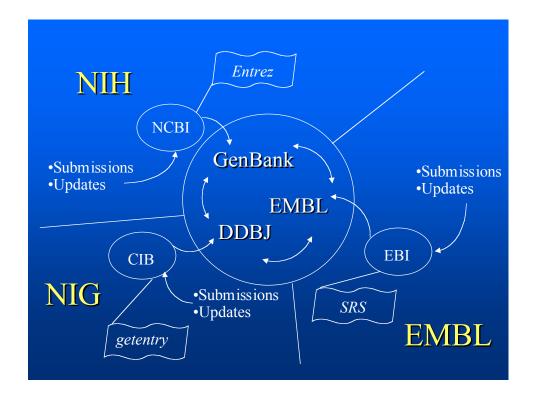
# **Primary Data**

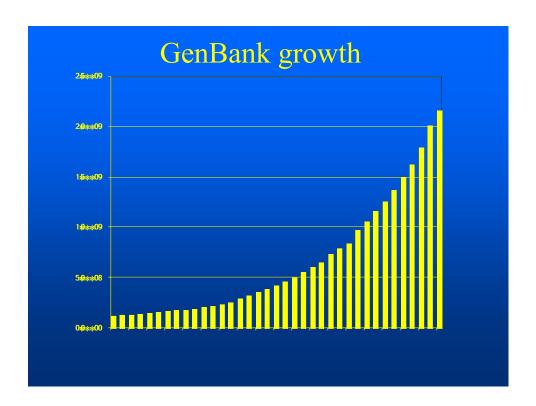
- DNA/RNA and protein sequences are the primary data for computational biology.
- Understanding the various types sequences present in GenBank is key to any interpretation in computational biology.
- Also understand that, as careful as NCBI and others are, errors do creap in, and always keep that critical eye open.

#### What is GenBank?

GenBank is the NIH genetic sequence database of all publicly available DNA and derived protein sequences, with annotations describing the biological information these records contain.

http://www.ncbi.nlm.nih.gov/Web/Genbank/index.html Benson *et al.*, 1999, *Nucleic Acids Res.* **27**:12-17





### GenBank - Release 110 - Dec 1998

> 50,000 "species" or "terminal nodes" 3,043,729 entries or GBFF 2,162,067,871 nucleotides

- •Full release of GenBank every 2 months.
- •Incremental and cumulative releases: daily.
- •GenBank is only available from the Internet.

#### GenBank Release 110 top 10 Records Bases **Species** 1,084,135,266 Homo sapiens 1,676,931 416,959 200,554,172 Mus musculus 76,740 147,625,327 Caenorhabditis elegans Arabidopsis thaliana 71,363 82,625,620 67,852 72,625,772 Drosophila melanogaster 46,235 Rattus norvegicus 28,943,917 10,595 28,693,027 Saccharomyces cerevisiae 56,789 28,385,949 Oryza sativa 52,126 22,002,992 Rattus sp. 4,972 18.095.876 Escherichia coli 32,195 16,636,036 Fugu rubripes

# **Organismal Divisions**

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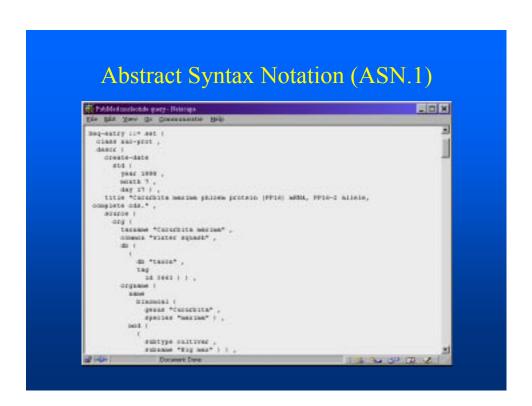
BCT	Bacterial	DDBJ - GenBank
FUN	Fungal	EMBL
HUM	Homo sapiens	DDBJ - EMBL
INV	Invertebrate	all
MAM	Other mammalian	all
ORG	Organelle	EMBL
PHG	Phage	all
PLN	Plant	all
PRI	Primate	all
PRO	Prokaryotic	EMBL
ROD	Rodent	all
RNA	Structural RNA	all
SYN	Synthetic and chimeric	all
VRL	Viral	all
VRT	Other vertebrate	all

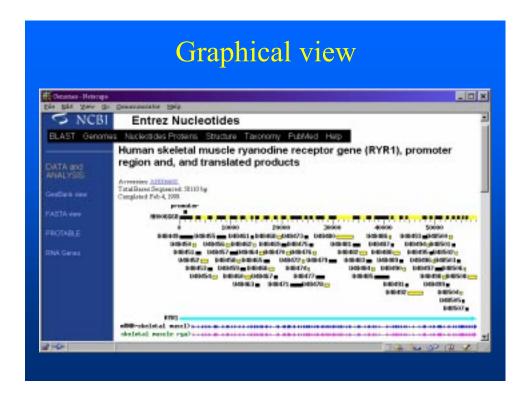
# **Guiding Principals**

- GenBank is a nucleotide-centric view of the information space.
- GenBank is a repository of all publicly available sequences.
- In GenBank, records are grouped for various reasons: understand this is key.
- Data in GenBank is only as good as what you put in: applying this is quite important.

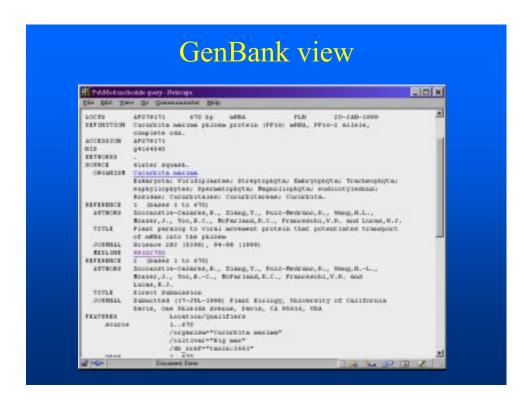
#### GBFF and ASN.1

- GenBank data is maintained at the NCBI as ASN.1
- ASN.1 is a language that is used by computers to store, maintain, validate and show sequence information.
- the GenBank Flat File (GBFF) is one of these views (report) but has taken a life of its own.









```
LOCUS HSU40282 1786 bp mRNA PRI 28-NOV-1997
DEFINITION Homo sapiens integrin-linked kinase (ILK) mRNA, complete cds.
ACCESSION U40282
NID g2648173
KEYWORDS .
SOURCE human.
ORGANISM Homo sapiens
Eukaryotae; mitochondrial eukaryotes; Metazoa; Chordata;
Vertebrata; Eutheria; Primates; Catarrhini; Hominidae; Homo.

REFERENCE 1 (bases 1 to 1786)
AUTHORS Hannigan,G.E., Leung-Hagesteijn,C., Fitz-Gibbon,L., Coppolino,M.G.,
Radeva,G., Filmus,J., Bell,J.C. and Dedhar,S.

TITLE Regulation of cell adhesion and anchorage-dependent growth by a new beta 1-integrin-linked protein kinase

JOURNAL Nature 379 (6560), 91-96 (1996)
MEDLINE 96135142
REFERENCE 2 (bases 1 to 1786)
AUTHORS Dedhar,S. and Hannigan,G.E.
TITLE Direct Submission
JOURNAL Submitted (07-NOV-1995) Shoukat Dedhar, Cancer Biology Research, Sunnybrook Health Science Centre and University of Toronto, 2075
Bayview Avenue, North York, Ont. M4N 3M5, Canada
```

# Sample GenBank Record: part 2

```
Source 1..1786
/organism="Homo sapiens"
/db_xref="taxon:9606"
/chromosome="11"
/map="1p15"
/cell_line="HeLa"

gene 1..1786
/gene="ILK"

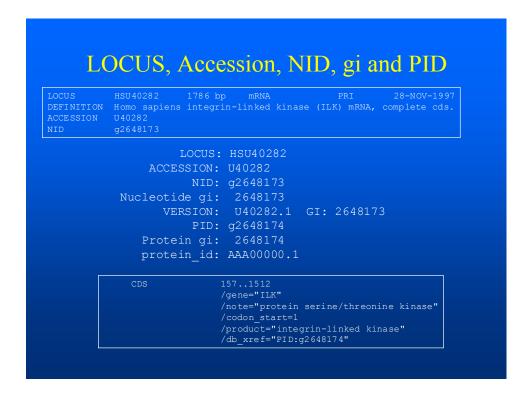
CDS 157..1512
/gene="ILK"
/note="protein serine/threonine kinase"
/codon_start=1
/product="integrin-linked kinase"
/db_xref="PID:g2648174"
/translation="MDDIFTQCREGNAVAVRLWLDNTENDLNQGDDHGFSPLHWACRE GRSAVVEMLIMRGARINVMNRGDDTPLHLAASHGHRDIVQKLLQYKADINAVNEHGNV
... <deleted lines>
BASE COUNT 443 a 486 c 479 g 378 t

ORIGIN

1 gaattcatct gtcgactgct accacgggag ttccccggag aaggatcctg cagcccgagt
... <deleted lines>
//
```

#### LOCUS, Accession, NID, gi and PID

```
LOCUS: Unique string of 10 letters and numbers in
     and is therefore a poor sequence identifier.
ACCESSION: A unique identifier to that record, citable
     entity; does not change when record is updated. A good
     record identifier, ideal for citation in publication.
NID: Nucleotide identifier: g, e or d prefix to gi number.
     Ideal identifier for specific version of a sequence.
Nucleotide gi: Geninfo identifier (gi), a unique integer
     which will change every time the sequence changes.
Accession.version: New system (expected late 1998) where
     the accession and version play the same function as
     the accession and gi number.
PID: Protein Identifier: g, e or d prefix to gi number.
Protein gi: Geninfo identifier (gi), a unique integer which
    will change every time the sequence changes.
protein id: new identifier which will have the same
     structure and function as the nucleotide Accession
     and version numbers.
```



# GenBank - Release 110

GB division	Nucleotides
Organisms	933,008,136
EST	762,208,762
HTG	218,559,327
GSS	208,046,224
PAT	40,245,422
STS	22,535,866

# GenBank Organismal divisions:

PRI - Primate	BCT - Bacterial
ROD - Rodent	RNA - Structural
<b>MAM</b> - Mammalian	<b>VRL</b> - Viral
<b>VRT</b> - Vertebrate	PHG - Phage
<b>INV</b> - Invertebrate	SYN - Synthetic
<b>PLN</b> - Plant	<b>UNA</b> - Unannotated

#### **Functional Divisions**

PAT - Patent

**EST** - Expressed Sequence Tags

**STS** - Sequence Tagged Sites

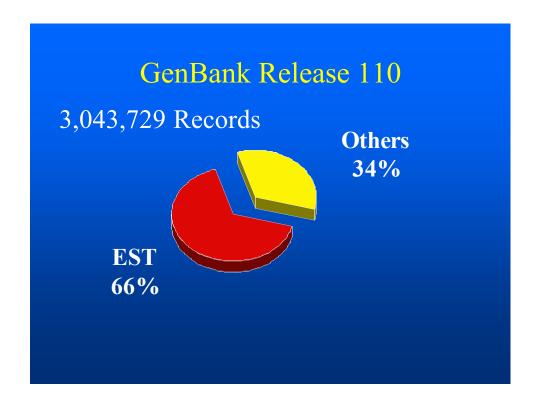
**GSS** - Genome Survey Sequences

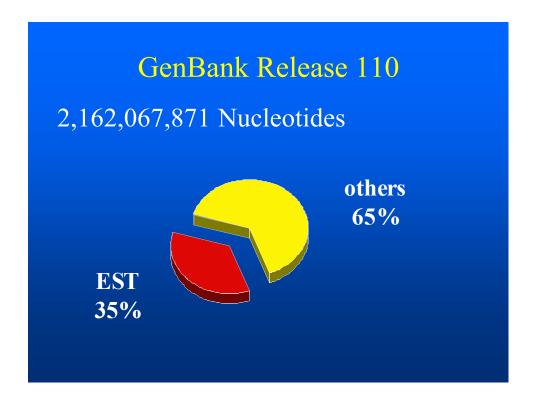
**HTG** - High Throughput Genome

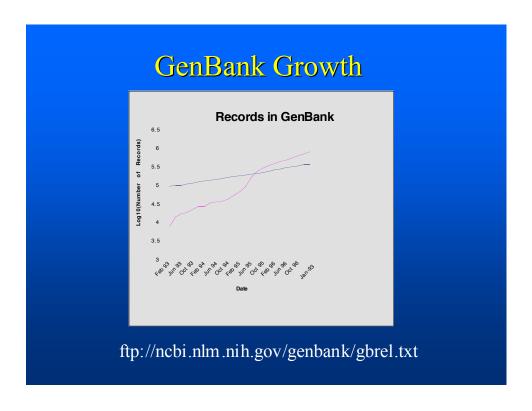
# EST: Expressed sequence Tag

Expressed sequence Tags are short (300-500 bp) single reads from mRNA (cDNA) which are produced in large numbers. They represent a snapshot of what is expressed in a given tissue, and developmental stage.

Also see: http://www.ncbi.nlm.nih.gov/dbEST/http://www.ncbi.nlm.nih.gov/UniGene/









#### STS

Sequenced Tagged Sites, are operationally unique sequence that identifies the combination of primer pairs used in a PCR assay that generate a maping reagent which maps to a single position within the genome.

Also see: http://www.ncbi.nlm.nih.gov/dbSTS/ http://www.ncbi.nlm.nih.gov/genemap/

#### **GSS**

Genome Survey Sequences are similar in nature to the ESTs, except that its Sequences are genomic in origin, rather than cDNA (mRNA).

The GSS division contains:

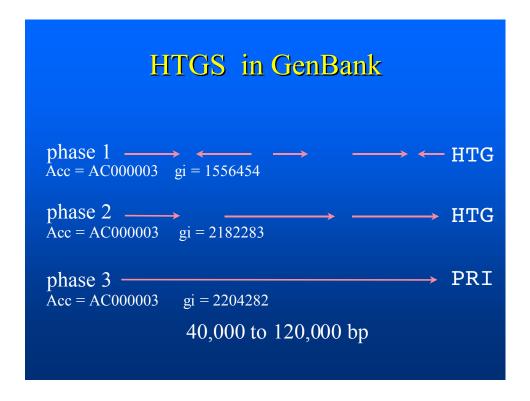
- random "single pass read" genome survey Sequences.
- single pass reads from cosmid/BAC/YAC ends (these could be chromosome specific, but need not be)
- exon trapped genomic Sequences
- Alu PCR Sequences

Also see: http://www.ncbi.nlm.nih.gov/dbGSS/

### HTG: High Throughput Genome

High Throughput Genome Sequences are unfinished genome sequencing efforts records. Unfinished records have gaps in the nucleotides sequence, low accuracy, and no annotations on the records.

Also see: http://www.ncbi.nlm.nih.gov/HTGS/ Ouellette and Boguski (1997) Genome Res. 7:952-955



```
LOCUS HSAC000003 120000 bp DNA HTG 20-SEP-1996
DEFINITION *** UENCING IN PROGRESS *** Chromosome 17 genomic sequence; HTGS phase 1, 6 unordered pieces.

ACCESSION AC000003
REYWORDS HTG; HTGS_PHASE1.
...

COMMENT 

*** WARNING: Phase 1 High Throughput Genome sequence ***

*** *** * This sequence is unfinished. It consists of 6 contigs for * which the order is not known; their order in this record is * arbitrary. In some cases, the exact lengths of the gaps * between the contigs are also unknown; these gaps are presented * as runs of N as a convenience only. When uencing is complete, * the sequence data presented in this record will be replaced *by a single finished sequence with the same accession number.

* 1 22526: contig of 22526 bp in length * 22036 33919: contig of 10884 bp in length * 23036 33919: contig of 10884 bp in length * 34428 61877: contig of 27450 bp in length * 34428 61877: contig of 27450 bp in length
```

```
LOCUS ACOOOOO3 122228 bp DNA PRI 07-OCT-1997
DEFINITION Homo sapiens chromosome 17, clone 10 4H12, complete sequence.
ACCESSION ACOOOOO3
NID g2204282
KEYWORDS HTG.
SOURCE human.
...

COMMENT The Staden databases, finishing information, and all chromatographic files used in the assembly of this clone are available from our anonymous ftp site.

All repeats were identified using RepeatMasker: Smit, A.F.A. & Green, P. (1996-1997)
http://ftp.genome.washington.edu/RM/RepeatMasker.html.

FEATURES Location/Qualifiers
source 1..122228
/organism="Homo sapiens"
/db_xref="taxon:9606"
/clone="104H12"
/clone lib="Research Genetics/Cal Tech CITB978SK-B (plates 1-194)"
/chromosome="17"
repeat_region 261..370
/rpt_family="MLT1B"
```

# **Guiding Principals**

In GenBank, records are grouped for various reasons, be it in organismal or functional divisions: understanding this is key to being able to fully exploit this database.

### Why submit sequences to GenBank?

- No longer submit Sequences to Journal
- Journal scanning is no longer taking place
- Electronic format more useful and allows validations
- Sequences sent to DDBJ/EMBL/GenBank are exchanged daily
- Best way to exchange new data, and updates

#### What to submit?

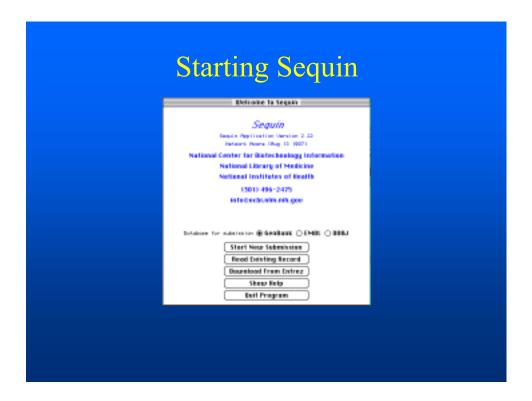
- DNA sequence atcttgctggggctgggtgaccaa
- Where it comes from: The BioSource
  mouse mRNA from gene on chromosme 9
- The citation, if one is present. The Cit-Sub
- What is relevent about this sequence? The annotated features.
- Other important Sequences: encodes protein which has function Y in mouse development.

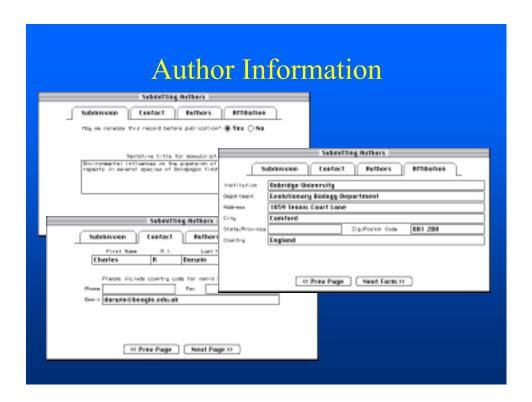
#### Which Tool?

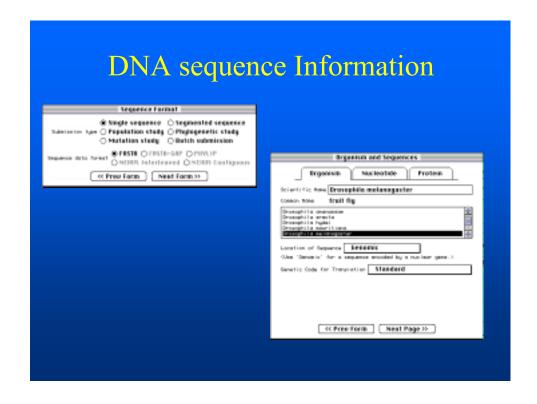
- BankIt: Web based tool which is simple, easy to use, great for simple submissions, but not ideal for complicated ones.
- Sequin: Client that you need to d/l to your computer, a little harder to learn, but has great documentation, and ideal for complicated, large, multiple submissions.

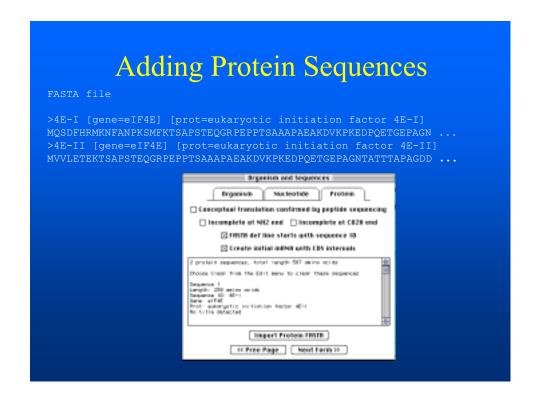
# Sequin

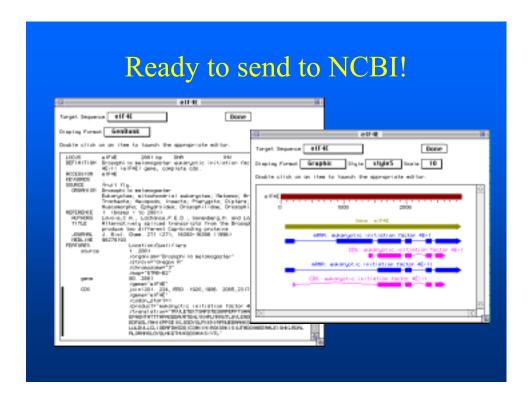
- http://www.ncbi.nlm.nih.gov/Sequin/
- Sequence editor for new submissions orr updates
- multi-platform (Mac/PC/Unix)
- built-in validation suite
- can do: segmented sets pop/phylo sets
  - large records different views
  - specialized editors
  - complex or simple annotations
  - BLAST and Entrez client

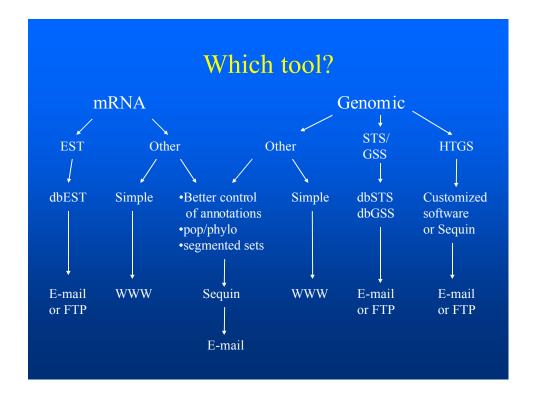






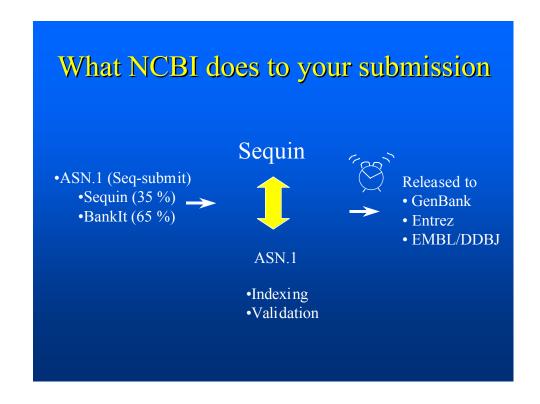






### Where to Submit?

- Sequin files are e-mailed to: gb-sub@ncbi.nlm.nih.gov
- BankIt: http://www.ncbi.nlm.nih.gov/BankIt/
- EST/GSS/STS send e-mail to: batch-sub@ncbi.nlm.nih.gov
- HTGS send query e-mail to: htgs@ncbi.nlm.nih.gov
- Not sure? e-mail to: info@ncbi.nlm.nih.gov

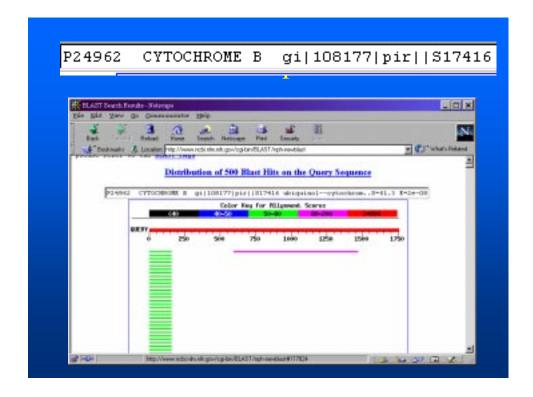


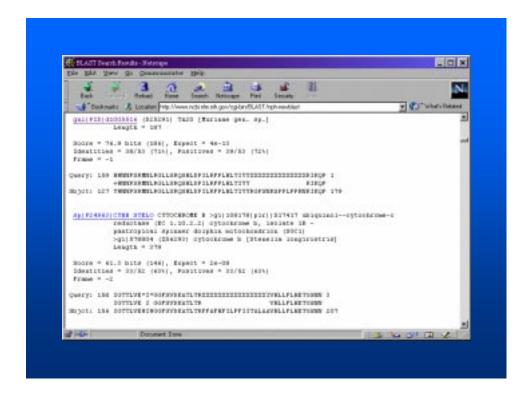
#### What is validated?

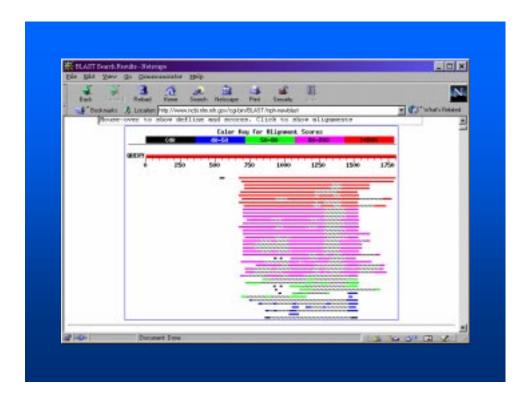
- Biological Validations
  - gene namedivision
  - protein namesDEFINITION line
- Computed validations
  - TaxonomySplice sites
  - CDS translateSpelling
  - Citation
  - BLASTX/BLASTN (vector, duplications, updates)

# Updates -- the 4 "W"

- Who updates?
  Submitters, Journals, "3rd party"
- What to update?Gene names, citations, new product, sequencing errors
- Where? update@ncbi.nlm.nih.gov
- Why update?







From francis@cmmt.ubc.ca Wed Mar 3 22:32:19 1999
To: ddbjupdt@ddbj.nig.ac.jp
Subject: D25291 mito

Dear colleagues,

it appears that DDBJ record D25291 is contaminated with mitochondrial Sequences from nucleotide 673 to 1803, as it is identical to mouse mitochondrial sequence (EMBL V00711) for more than 1100 nucleotides.

I would recommend deleting that segment of the record, or removing the record altogether, as it leads to unfortunate misinterpretation of the data when using GenBank or DDBJ. The protein sequence (which is erroneous, as it is all of mitochondrial origin) should definitely be removed as well.

# Sequence Updated

LOCUS MUSNGH 1803 bp mRNA ROD 29-AUG-199
DEFINITION Mouse neuroblastoma and rat glioma hybridoma cell line NG108-1
cell TA20 mRNA, complete cds.

ACCESSION D25291
NID g1850791
VERSION D25291.1 GI:1850791

LOCUS MUSNGH 619 bp mRNA ROD 12-MAR-1999
DEFINITION Mouse neuroblastoma and rat glioma hybridoma cell line NG108mRNA.

ACCESSION D25291
NID g9999999
VERSION D25291.2 GI:9999999

# **Guiding Principals**

Data in GenBank is only as good as what you put in: applying and ensuring this (in an active, day too day fashion) will only make everybody's work that much more easier ...

### **Summary**

- GenBank is a nucleotide-centric view of the information space, and is a report from the underlying ASN.1 data.
- In GenBank, records are grouped for various reasons: understand this is key to taking full advantage of this information.
- Sequin and BankIt can be used for updates and new submissions.
- Understanding the data elements in GenBank records is important, and allows you to take full control of the info.
- Data in GenBank is only as good as what you put in: applying this is crucial to the task at hand, dealing with the Everest of information before us.

# Acknowledgments

**GenBank Coordinator:** Ilene Mizrachi **GenBank Annotation Staff:** 

Gabriella Ryan Adams, Medha Bhagwat, Lori Black, Larry Chlumsky, Karen Clark, Irene Fang, Michael Fetchko, Pamela Jacques, Anthony Jung, Junga Kim, Jaime Kolonay, Pierre Ledoux Daniel Lyman, Baishali Maskeri, Jenny McDowell, Richard McVeigh, Lillian Riddick, Leigh Riley, Susan Schafer, Jane Weisemann, and Linda Yankie